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Requester's Full Name: JANE ZAKA Examiner #: 7751 Date: 7/11/05
Art Unit: 1635 Phone Number: 2-2765 Serial Number: 09/788,074
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Title of Invention: Method of Digging Arable Plots
Inventors (please provide full names): HOTA MISLIGIL, GS

Earliest Priority Date: 2/16/01

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Set	Items	Description
S1	668	AU=(HOTAMISLIGIL G? OR HOTAMISLIGIL, G?)
S2	448	S1 AND (DIABET? OR INSULIN)
S3	5	S2 AND MAL
S4	3	RD (unique items)

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S3	5	S2 AND MAL
S4	3	RD (unique items)

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0014189746 BIOSIS NO.: 200300148465
 Role of the fatty acid binding protein mall in obesity and **insulin** resistance.
 AUTHOR: Maeda Kazuhisa; Uysal K Teoman; Makowski Liza; Gorgun Cem Z; Atsumi Genichi; Parker Rex A; Bruning Jens; Hertzfel Ann Vogel; Bernlohr David A;
Hotamisligil Gokhan S (Reprint
 AUTHOR ADDRESS: Harvard School of Public Health, 665 Huntington Ave.,
 Boston, MA, 02115, USA**USA
 AUTHOR E-MAIL ADDRESS: ghotamis@hsph.harvard.edu
 JOURNAL: Diabetes 52 (2): p300-307 February 2003 2003
 MEDIUM: print
 ISSN: 0012-1797 (ISSN print)
 DOCUMENT TYPE: Article
 RECORD TYPE: Abstract
 LANGUAGE: English

Role of the fatty acid binding protein mall in obesity and **insulin** resistance.

...AUTHOR: ***Hotamisligil Gokhan S***

ABSTRACT: The metabolic syndrome is a cluster of metabolic and inflammatory abnormalities including obesity, **insulin** resistance, type 2 ***diabetes***, hypertension, dyslipidemia, and atherosclerosis. The fatty acid binding proteins aP2 (fatty acid binding protein (FABP)-4) and mall (FABP5) are closely related and both are expressed in adipocytes. Previous studies in aP2-deficient mice have indicated a significant role for aP2 in obesity-related **insulin** resistance, type 2 ***diabetes***, and atherosclerosis. However, the biological functions of mall are not known. Here, we report the generation of mice with targeted null mutations in the mall...

...address the role of the second adipocyte FABP in metabolic regulation in the presence and deficiency of obesity, absence of mall resulted in increased systemic **insulin** sensitivity in two models of obesity and ***insulin*** resistance. Adipocytes isolated from mall-deficient mice

also exhibited enhanced **insulin**-stimulated glucose transport capacity. In contrast, mice expressing high levels of mall in adipose tissue display reduced systemic ***insulin*** sensitivity. Hence, our results demonstrate that mall modulates adipose tissue function and contributes to systemic glucose metabolism and constitutes a potential therapeutic target in ***insulin*** resistance.

...REGISTRY NUMBERS: ***insulin***

DESCRIPTORS:

CHEMICALS & BIOCHEMICALS: ***insulin*** --...

... ***mal*** -1 fatty acid binding protein...

... ***insulin*** resistance development role, obesity development role, adipose tissue function modulatory role, systemic glucose metabolism role

4/3,K/2 (Item 1 from file: 399)

DIALOG(R)File 399:CA SEARCH(R)

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135200396 CA: 135(14)200396b PATENT

Treatment lipid metabolic disorders by inhibiting Mall, the keratinocyte lipid binding protein activity

INVENTOR(AUTHOR): Hotamisligil, Gokhan S.

LOCATION: USA

ASSIGNEE: President and Fellows of Harvard College

PATENT: PCT International ; WO 200160384 A1 DATE: 20010823

APPLICATION: WO 2001US5019 (20010216) *US PV183106 (20000217)

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4/3,K/3 (Item 1 from file: 149)

DIALOG(R)File 149:TGG Health&Wellness DB(SM)

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01863324 SUPPLIER NUMBER: 56456896 (USE FORMAT 7 OR 9 FOR FULL TEXT)

Altered **Insulin** Secretion Associated With Reduced Lipolytic

Efficiency in (aP2.sup.-/-) Mice.

Scheja, Ludger; Makowski, Liza; Uysal, K. Teoman; Wiesbrock, Sarah M.; Shimshek, Derya R.; Meyers, Daniel S.; Morgan, Maureen; Parker, Rex A.;

Hotamisligil, Gokhan S.

Diabetes, 48, 10, 1987

Oct,

1999

PUBLICATION FORMAT: Magazine/Journal; Refereed ISSN: 0012-1797

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WORD COUNT: 8092 LINE COUNT: 00671

Altered **Insulin** Secretion Associated With Reduced Lipolytic

Efficiency in (aP2.sup.-/-) Mice.

... ***Hotamisligil, Gokhan S.***

TEXT:

...fatty acid-binding protein (aP2) results in minor alterations of plasma lipids and adipocyte development but provides significant protection from dietary obesity-induced hyperinsulinemia and ***insulin*** resistance. To identify potential mechanisms responsible for this phenotype, we examined lipolysis and ***insulin*** secretion in (aP2.sup.-/-) mice. (Beta)-Adrenergic stimulation resulted in a blunted rise of blood glycerol levels in (aP2.sup.-/-) compared with (aP2.sup.+/+) mice...

...The decreased lipolytic response seen in the (aP2.sup.-/-) mice was not associated with altered expression levels of hormone-sensitive lipase or perilipin. The acute ***insulin*** secretory response to (Beta)-adrenergic stimulation was also profoundly suppressed in (aP2.sup.-/-) mice despite comparable total concentrations and only minor changes in the composition

...reduction in both stearic and cis-11-eicosenoic acids and an increase in palmitoleic acid were observed. The response of (aP2.sup.-/-) mice to other **insulin** secretagogues such as arginine and glyburide was similar to that of (aP2.sup.+/+) mice, arguing against generally impaired function of pancreatic (Beta)-cells. Finally, no...

...an adipo-pancreatic axis, the proper action of which relies on the presence of aP2. Consequently, aP2's role in the pathogenesis of type 2 **diabetes** might involve regulation of both hyperinsulinemia and **insulin** resistance through its impact on both lipolysis and ***insulin*** secretion. ***Diabetes*** 48:1987-1994, 1999

... aP2.sup.-/- animals were placed on a high-fat diet to induce obesity (14). The obese (aP2.sup.-/-) mice were significantly protected from hyperinsulinemia and **insulin** resistance compared with their wild-type ((aP2.sup.+/+)) littermates. This protection from dietary obesity-induced **insulin** resistance in the absence of aP2 suggests that adipocyte fatty acid metabolism is a critical component of the mechanisms leading to systemic ***insulin*** resistance in obesity.

Among potentially relevant parameters of adipocyte fatty acid metabolism, the lipolytic breakdown of stored triglycerides is of particular interest. Major physiologic regulation...

...release from adipose tissue are commonly increased in obesity. This increase has been suggested to be an important factor in the initiation of hyperinsulinemia and ***insulin*** resistance (23). In support of this hypothesis, experimental alterations in FFA metabolism have been demonstrated to influence both **insulin** secretion and peripheral ***insulin*** action (24-27). The release of fatty acids from adipocytes could involve FABP activity at several stages, including the efficiency of flux from the lipid droplet to the plasma membrane or intermediary metabolic activities. Hence, we examined the effects of aP2 deficiency on adipocyte lipolysis and the subsequent acute **insulin** secretory response. Our results demonstrate that aP2 plays an important role in regulation of both these parameters.

RESEARCH DESIGN AND METHODS

Animals and experimental conditions...

...in aliquots and stored at -20 (degrees) C. Enzymatic assays were used for the determination of serum FFAs (Wako, Richmond, VA) and glycerol (Sigma). Serum ***insulin***, C-peptide, and leptin levels were measured using radioimmunoassays from Linco Research (St. Charles, MO). Glucose levels were determined using blood glucose strips (MediSense, Waltham... analysis as described (33). The cDNA clones used for the generation of labeled probes (HSL, perilipin) were provided by Dr. C. Londos, National Institute of **Diabetes** and Digestive and Kidney Diseases, National Institutes of Health. A human ribosomal protein probe (36B4) (34) was used as loading control.

Reverse transcription-polymerase chain...

...mice. Lipolysis is an adipocyte function likely to be altered in (aP2.sup.-/-) mice and also a factor that might contribute to the protection from ***insulin*** resistance observed in these animals. To test this hypothesis in (aP2.sup.-/-) and (aP2.sup.+/+) mice, we first examined lipolysis in vivo using intraperitoneal administration...
 ...tissue (40-42). In a subset of experiments, we also used isoproterenol (10 mg/kg), a general (Beta)-adrenergic agonist. Circulating levels of FFAs and **insulin** have been shown to be maximally elevated between 10 and 15 min after intraperitoneal administration of CL 316,243 (43) (see below). In line with...

...basal was lower in the (aP2.sup.-/-) mice.

(Figure 1 ILLUSTRATION OMITTED)

In the postprandial state, lipolysis is suppressed because of the negative action of ***insulin*** (44,45). We next tested whether an attenuation of lipolysis could also be detected in the (aP2.sup.-/-) mice under this physiologic condition by performing...

...As expected, basal FFA and glycerol levels in the postprandial state were decreased compared with resting animals, consistent with the observed elevated basal levels of ***insulin***. The basal levels of both glycerol and FFAs were similar in (aP2.sup.-/-) and (aP2.sup.+/+) animals (Table 1). After administration of CL 316,243...

mmol/l)	0.31 (+ or -)	0.06	0.98 (+ or -)	0.09
Glycerol (mmol/l)	0.18 (+ or -)	0.01	0.42 (+ or -)	0.02
Insulin (ng/ml)	3.2 (+ or -)	0.96	8.4 (+ or -)	2.4
Glucose (mg/dl)	174 (+ or -)	6.0	148 (+ or -)	9.8
Leptin (ng...				

...mmol/l)	0.26 (+ or -)	0.04	0.80 (+ or -)	0.08
Glycerol (mmol/l)	0.18 (+ or -)	0.03	0.35 (+ or -)	0.02(*)
Insulin (ng/ml)	2.4 (+ or -)	0.39	3.3 (+ or -)	0.9(*)
Glucose (mg/dl)	161 (+ or -)	8.2	169 (+ or -)	15.1
Leptin (ng...				

...gel electrophoresis was used to determine expression of aP2 and mall in (aP2.sup.-/-) and (aP2.sup.+/+) adipocytes (data not shown).

(Figure 3 ILLUSTRATION OMITTED)

Insulin secretion. (Beta)-Adrenergic stimulation in vivo is known to be accompanied by acute secretion of ***insulin*** (47,48). The mechanism of (Beta)-AR-mediated **insulin** secretion is not fully understood. Recent studies have shown that a major part of this secretory response is mediated by (Beta)-ARs on adipocytes without direct stimulation of pancreatic (Beta)-cells (43). Because chronically increased **insulin** secretion has been proposed to contribute to the development of ***insulin*** resistance (23) and (aP2.sup.-/-) mice are protected from hyperinsulinemia and **insulin** resistance (14), we wondered whether alterations exist in the **insulin** secretory response to (Beta)-agonists in these animals. Indeed, we observed a profoundly blunted ***insulin*** response to CL 316,243 in (aP2.sup.-/-) mice investigated in the resting state. Whereas the basal ***insulin*** levels were similar in both groups, stimulated ***insulin*** levels of (aP2.sup.-/-) mice (7.5 (+ or -) 1.05 ng/ml) reached only 39% of the levels seen in (aP2.sup.+/+) controls (19.4...

...3 vs. 150 (+ or -) 7.3 mg/dl, (aP2.sup.+/+) vs. (aP2.sup.-/-); n = 13).

An even stronger effect was observed after administration of isoproterenol.

Insulin levels rose to 23.3 (+ or -) 2.4 ng/ml in (aP2.sup.+/+) mice but only to 5.7 (+ or -) 0.9 ng/ml in...

...0001) (Fig. 4). This diminished response in the (aP2.sup.-/-) mice was also reflected in systemic levels of C-peptide, a more accurate indicator

of ***insulin*** secretion than ***insulin*** itself. While basal levels were equal in both genotypes (352 (+ or -) 26 vs. 370 (+ or -) 18 pmol/l in (aP2.sup.-/-) vs. (aP2.sup.+/+) animals...

...0.005; n = 7) after administration of CL 316,243.

(Figure 4 ILLUSTRATION OMITTED)

One potential cause for the observed attenuation of (Beta)-AR-mediated ***insulin*** secretion in (aP2.sup.-/-) mice is a general reduction in pancreatic (Beta)-cell activity. To test this hypothesis, we performed **insulin** secretion experiments using alternative **insulin** secretagogues including arginine (1 g/kg) or a combination of arginine and the sulfonylurea glyburide (0.2 mg/kg). After intraperitoneal administration of arginine, systemic **insulin** concentrations were similar in (aP2.sup.-/-) and (aP2.sup.+/+) mice (4.7 (+ or -) 0.41 vs. 6.1 (+ or -) 0.94 ng/ml) (Fig. 4...

...levels in (aP2.sup.-/-) and (aP2.sup.+/+) mice (16.6 (+ or -) 1.2 vs. 16.1 (+ or -) 1.6 ng/ml) (Fig. 4).

Because altered **insulin** secretion might have additional effects on physiologic parameters in the postprandial period compared with the resting period, we also investigated (Beta)-AR-mediated **insulin** secretion in mice under this metabolic condition. As expected, basal

insulin levels were elevated in postprandial animals (Table 1). On injection of CL 316,243, blood ***insulin*** levels rose in (aP2.sup.-/-) and (aP2.sup.+/+) mice (Table 1). However, significantly higher

insulin concentrations were observed in (aP2.sup.+/+) compared with (aP2.sup.-/-) mice (8.4 (+ or -) 2.4 vs. 3.3 (+ or -) 0.9 ng/ml; P (is less than) 0.05).

Systemic concentrations of **insulin** secretagogues in response to (Beta)3-adrenergic stimulation. Experimental elevation of systemic FFAs has been shown to stimulate **insulin** secretion in vivo, making FFAs likely candidates for adipose-derived **insulin** secretagogues associated with (Beta)3-adrenergic stimulation. Total FFA levels appeared not to be responsible, however, for the difference in **insulin** secretion between the genotypes in our experiments where, in the resting state, systemic FFA levels were equal or even higher in (aP2.sup.-/-) vs. (aP2.sup.+/+) mice (Fig. 1B). Therefore, we tested whether the observed suppression of **insulin** secretion might be due to altered blood levels in (aP2.sup.-/-) mice of other known secretagogues. To determine a kinetic profile of **insulin** and these secretagogues, blood was collected from untreated animals and 5, 10, or 15 min after intraperitoneal administration of CL 316,243. As shown in Fig. 5A, ***insulin*** began to rise 5 min after (Beta)3-adrenergic stimulation. The average blood ***insulin*** concentration in the (aP2.sup.-/-) mice was already significantly lower at that point compared with the (aP2.sup.+/+) controls. ***Insulin*** continued to increase up to 15 min after injection, where the difference between the genotypes was most striking (Fig. 5A). Basal blood glucose levels were...

...Fig. 5B). After (Beta)3-adrenergic stimulation, no significant difference in blood glucose levels was evident between the two genotypes (Fig. 5B), despite strikingly different ***insulin*** concentrations, suggesting a difference in ***insulin*** sensitivity. Basal FFA concentrations were moderately but significantly higher in (aP2.sup.-/-) compared with (aP2.sup.+/+) mice (0.46 (+ or -) 0.03 vs. 0.32...

...CL 316,243 and increased only slightly thereafter, with no significant difference between the genotypes (Fig. 5C). Recent studies have shown that leptin can suppress ***insulin*** secretion (49-53). To test a potential role of leptin, we also measured serum leptin levels before and after the injection of CL 316,243...

...the experiment (Fig. 5D).

(Figure 5 ILLUSTRATION OMITTED)

Recently published data indicate that the endogenous subtypes of fatty acids differ in their potential to stimulate **insulin** secretion from pancreatic (Beta)-cells (54). Since absence of aP2 might change the composition, and therefore the insulinotropic potency, of FFAs released from adipocytes, we...

...Omega9) (30% decrease in (aP2.sup.-/-); P (is less than) 0.05).

(Figure 6 ILLUSTRATION OMITTED)

aP2 expression in pancreatic (Beta)-cells. Because the *****insulin***** secretion pattern in (aP2.sup.-/-) mice is significantly altered, it is critical to determine whether the aP2 gene is expressed in pancreatic islets. Previous studies...

...HNF4(Alpha), a gene known to be expressed in these cells.

(Figure 7 ILLUSTRATION OMITTED)

DISCUSSION

In this study, we examined lipolysis and the associated **insulin** secretion in mice lacking aP2 and their wild-type counterparts. Experiments performed under various physiologic conditions consistently demonstrated an attenuated rise of glycerol in the...sup.-/-) adipocytes.

Stimulation of lipolysis with 6-AR-specific agonists such as CL 316,243 and isoproterenol is accompanied by an acute increase in blood *****insulin***** (47,48,62). Surprisingly, this *****insulin***** response was profoundly reduced in (aP2.sup.-/-) mice (Fig. 4; Table 1). A similar reduction of C-peptide levels in (aP2.sup.-/-) mice after (Beta)-adrenergic stimulation indicates that the defect is, at least partially, at the level of *****insulin***** secretion. This decreased responsiveness is unlikely to be through an indirect mechanism such as generally diminished activity of pancreatic (Beta)-cells (63-65), since arginine or a combination of arginine and glyburide stimulated **insulin** secretion to a similar extent in (aP2.sup.-/-) and (aP2.sup.+/+) mice (Fig. 4). Also, the reduced response is probably not related to glucose, which is a weak **insulin** secretagogue in the mouse strain used (C57B1/6) (66). We also tested whether a previously unrecognized expression of aP2 in pancreatic islets might be a...

...excluding the possibility of a direct impact of aP2 deficiency at that site.

Previous studies have suggested that the signal for (Beta)-AR stimulation of **insulin** secretion originates from adipocytes (43) and that it involves fatty acids (25,54,67,68). However, the strikingly different *****insulin***** response seen in the (aP2.sup.-/-) mice in our study occurred under experimental conditions in which significant differences in blood concentrations of total FFAs compared...

...controls were not evident (Fig. 5). This prompted us to examine specific fatty acids, since recently published experiments using perfused rat pancreas showed that the **insulin** secretory potential of individual fatty acid species is quite different, increasing with length and saturation of the hydrocarbon chain (54). In our experiments, we did...

...11-Eicosenoic acid has not been determined. Therefore, it is not clear whether these alterations are physiologically relevant and might account for the profoundly reduced *****insulin***** response in (aP2.sup.-/-) mice.

In this study, we observed decreased release of both glycerol and FFAs in response to dibutyryl cAMP stimulation in (aP2...

...Regardless, it is clear from the data shown here that under conditions where systemic FFA levels are similar, a dramatic difference is still detectable in **insulin** secretion in response to lipolytic stimuli in aP2-deficient animals. While it is possible to argue that the net local FFA input to the pancreatic possibilities emerge in view of the observed impact

of aP2 deficiency on ***insulin*** secretion. First, these results provide strong support for the notion of an endocrine axis between adipose tissue and the pancreas, demonstrating that a specific and isolated defect of adipose tissue can have a profound effect on the latter organ. Second, since hyperinsulinemia can lead to peripheral **insulin** resistance, (aP2.sup.-/-) animals might be protected from the development of obesity-induced **diabetes**, at least in part, through a reduction in ***insulin*** secretion triggered by adipocyte products. Currently, the mechanisms underlying increased peripheral **insulin** sensitivity in the absence of aP2 are not fully understood. Further characterization of these pathways might therefore provide important insights into the pathophysiology of type 2 **diabetes** and facilitate the development of novel therapeutic targets.

Note added in proof. During the review of this manuscript, Coe et al. (J Lipid Res 40...

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